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Interleukin-18 in Intestinal Inflammation: Friend and Foe?

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Genetic studies and other experimental data have linked inflammatory bowel diseases with inflammasome activation. In this issue of *Immunity*, Zaki et al. (2010) and Dupaul-Chicoine et al. (2010) provide a detailed characterization of the regulatory task of the inflammasome in intestinal epithelial cells.

Activation of the NLRP3 inflammasome results in the conversion of pro-caspase-1 to caspase-1 followed by the cleavage of pro-interleukin-1β (pro-IL-1β) and pro-IL-18 to their active cytokines. Gain-of-function mutations within *NLRP3* have been associated with various autoinflammatory disorders, such as the Muckle-Wells syndrome (MWS), familial cold autoinflammatory syndrome (FCAS), and neonatal-onset multisystem inflammatory disease (NOMID). These hereditary periodic fever syndromes are characterized by skin rashes, periodic fever, and arthritis and are now lumped together and called cryopyrin-associated periodic fever syndromes (CAPS). Anti-IL-1β is uniquely effective in treating CAPS, thus emphasizing the central role of the NLRP3 (Dinarello, 2004). These findings led to the assumption that other autoinflammatory disorders such as inflammatory bowel diseases might equally be a consequence of NLRP3 inflammasome activation.

For inflammatory bowel diseases, first evidence was provided in 1999 by Pizarro et al. (1999). This study revealed an upregulation of IL-18 in the intestine of Crohn's disease patients with a higher expression in active disease and a localization of IL-18 synthesis in intestinal epithelial cells (IECs) as well as macrophages and dendritic cells (DCs) in the lamina propria. Immunoblot analysis confirmed that mature and thus active IL-18 and not the constitutively expressed precursor was present. Functional data followed this initial descriptive study indicating that mice were protected in various models of colitis, including dextran sulfate sodium (DSS)- and trinitrobenzene sulphonic acid (TNBS)-induced colitis, when either pro-IL-18 could not be activated or the active cytokine was neutralized via antibody (Kanai et al., 2001; Siegmund et al., 2001). In agreement with the human data, IL-18 production was localized to IECs and macrophages or DCs of the lamina propria. At that time, one could conclude that neutralization of IL-18 should be beneficial for intestinal inflammation. However, subsequently, the concept became questionable. Although blocking IL-18 in experimental models of colitis was effective, genetic data suggested that SNPs in the *NLRP3* region contribute to Crohn's disease susceptibility. The SNPs are associated with a decrease of *NLRP3* mRNA expression (Villani et al., 2009). In theory, a decrease of NLRP3 is followed by a decrease in NLRP3 inflammasome activation and consecutively a decrease in IL-1β and IL-18. Together these data indicated that the regulation of intestinal inflammation by the NLRP3 inflammasome is more complex. In fact, functional studies for intestinal inflammation focusing on the NLRP3 inflammasome were lacking. This issue of *Immunity* provides data that allow for a novel working hypothesis (Figure 1).

Zaki et al. (2010) in this issue of *Immunity* subjected *Nlrp3*$^{-/-}$ mice to experimental colitis with DSS, which resulted in increased mortality as well as macroscopic and histologic signs of colitis when compared to wild-type (WT) mice. Similar susceptibility was observed for mice deficient in the inflammasome component ASC (*Pycard*$^{-/-}$) and in caspase 1 (*Casp1*$^{-/-}$). Because the Nlrp3 inflammasome activation is prerequisite for either IL-1β or IL-18 activation, it is not surprising that IL-18 is upregulated at the site of inflammation in DSS-exposed WT but not in *Nlrp3*$^{-/-}$, *Pycard*$^{-/-}$, and *Casp1*$^{-/-}$ mice. A key role could be assigned to IL-18 because disease deterioration could be reversed by the administration of IL-18. To define the relevant cell population, bone marrow chimera were generated, indicating that nonhematopoietic cells are critical for the increased susceptibility. Considering these results in view of earlier data, the epithelial cells and thus the epithelial barrier are likely to play a crucial role. The critical function of the epithelial cells is the maintenance of the epithelial barrier. After DSS exposure, an increase in intestinal barrier permeability could be observed in *Nlrp3*$^{-/-}$ and *Casp1*$^{-/-}$ compared to WT mice by FITC-dextran. Similarly, bacterial translocation was elevated in these animals and furthermore, antibiotics treatment protected from DSS-induced morbidity and mortality. As a mechanism behind this defect, epithelial cell proliferation was higher in DSS WT than in DSS *Nlrp3*$^{-/-}$ and *Casp1*$^{-/-}$ mice, suggesting that Nlrp3 inflammasome activation of epithelial cells is followed by a compensatory proliferative response.

In the related study in this issue of *Immunity*, Dupaul-Chicoine et al. (2010) explore the control of intestinal homeostasis by inflammatory caspases, namely, caspase-1 and caspase-12. These authors also apply the model of DSS-induced colitis. During acute colitis, *Casp1*$^{-/-}$ mice are more susceptible whereas the *Casp12*$^{-/-}$ mice are comparable to DSS-exposed WT mice. Interestingly, *Pycard*$^{-/-}$ mice reflected an intermediate disease phenotype. Because the primary defect in acute DSS colitis is found in the epithelium, the epithelial integrity was evaluated. Although no difference in tight junction expression was revealed, cell proliferation in *Casp1*$^{-/-}$ mice was decreased whereas it was increased in *Casp12*$^{-/-}$ mice. Accordingly,

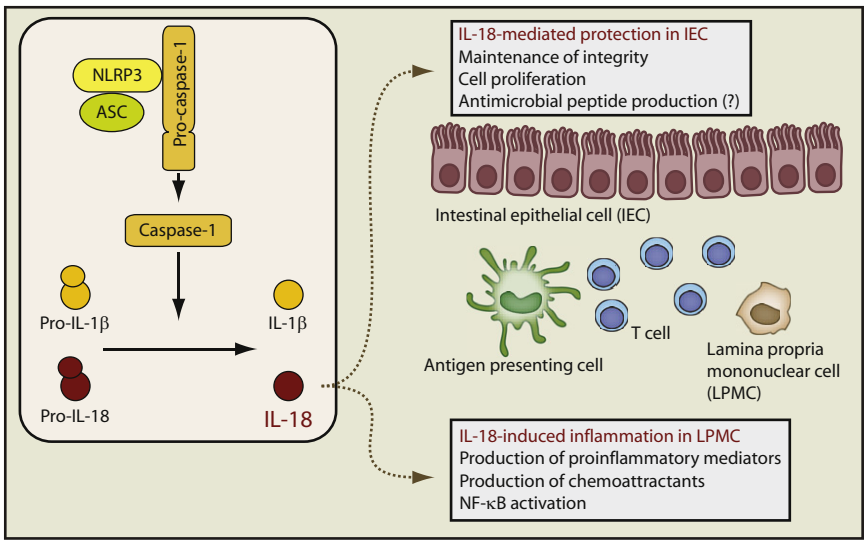

Figure 1. IL-18-Mediated Protective and Harmful Effects in Intestinal Inflammation
Depending on the site of activation, the release of IL-18 can present a mandatory protective mechanism or exert the key mediator for intestinal destruction. After activation within the intestinal epithelial cells (IEC), IL-18 contributes to the preservation of the intestinal barrier, mainly by inducing proliferation and thus enhancing the regeneration of the damaged epithelium. Whether or not IL-18 is involved in the regulation of antimicrobial peptides has not been addressed. In contrast, after activation in the lamina propria, IL-18 mainly in synergism with IL-12 exerts proinflammatory properties. This is followed by an infiltration of neutrophils as well as an activation of effector T cells, thus sustaining the inflammatory cascade.

FITC-dextran permeability as well as bacterial translocation was highest in *Casp1*$^{-/-}$ mice, whereas there was no difference between WT and *Pycard*$^{-/-}$ animals. The main IL-18-producing cell population in DSS WT mice are IECs. The protective role of IL-18 was underlined by exogenous IL-18 administration that resulted in protection of *Casp1*$^{-/-}$ mice from DSS colitis. In contrast to caspase-1, caspase-12 is a dominant negative regulator of caspase-1 and signaling by the intracellular sensor NOD. *Casp12*$^{-/-}$ mice showed a comparable phenotype to WT mice in the acute model of DSS colitis, but expressed an increased mortality when DSS exposure was prolonged to 15 days. Conflictingly, in the chronic DSS model they were protected compared to WT mice. In contrast, in the AOM-DSS model a dramatic tumor burden was observed in the *Casp12*$^{-/-}$ mice whereas only a few tumors were detected in the AOM-DSS WT group. This was associated with an overexpression of the survival factor Bcl-xl, an increase in cell proliferation, as well as more inflammation and an infiltration of macrophages, suggesting that the released mediators promote tumor growth. Accordingly, DNA microarray analysis of the tumors in *Casp12*$^{-/-}$ mice revealed an upregulation of proinflammatory as well as proliferative genes.

Both papers conclude that in their experimental setting IECs present the relevant IL-18-producing cell population. IL-18 is protective and furthermore required to maintain the epithelial integrity, thus preventing the translocation of bacteria and consequently death (Dupaul-Chicoine et al., 2010; Zaki et al., 2010). In the work from Zaki et al. (2010), *Nlrp3*$^{-/-}$ mice show a similar phenotype as *Casp1*$^{-/-}$ and *Pycard*$^{-/-}$ mice, suggesting that the Nlrp3 inflammasome activation represents the key step in this regulatory pathway. According to the study by Dupaul-Chicoine et al. (2010), an additional factor, namely caspase-12, is involved. Caspase-12 acts by inhibiting Nlrp3 activation. In the absence of caspase-12, the mice are comparable to WT mice in the acute DSS model; however, they are more susceptible to the sustained DSS exposure over 15 days. One could argue that an overactivation of the Nlrp3 inflammasome is rather deleterious. However, in the chronic DSS model, they are more protected but at the same time more prone to tumor development in the AOM-DSS model.

Additional literature underlines the concept that otherwise "proinflammatory" pathways are required for the epithelium to guarantee the intestinal immune homeostasis—acting as a physical barrier, separating luminal bacteria and immune cells, and also expressing antimicrobial peptides. For instance, epithelial cell-specific inhibition of NF-κB through conditional ablation of NEMO spontaneously caused severe chronic intestinal inflammation (Nenci et al., 2007). NF-κB deficiency led to apoptosis of IECs and impaired antimicrobial peptide expression and translocation of bacteria into the mucosa. Remarkably, deficiency of MyD88 in the same mice prevented the development of intestinal inflammation, indicating that in the lamina propria TLR activation by intestinal bacteria is essential for disease pathogenesis (Nenci et al., 2007).

The question remains whether IL-18 is comparable to NF-κB proinflammatory for the lamina propria. Recent genetic work together with earlier experimental data provide the answer and allow the establishment of a working model. Autophagy-related 16-like 1 (Atg16l1) has been implicated in the pathogenesis of Crohn's disease; however, how autophagy regulates inflammation was not resolved. Autophagy presents a bulk degradation system that controls the degradation of long-lived proteins, insoluble protein aggregates, and invading microbes. Mice lacking Atg16l1 solely in hematopoietic cells are not only highly susceptible to DSS-induced colitis but furthermore, neutralizing antibodies to either IL-1β or IL-18 in these mice resulted in an amelioration of disease (Saitoh et al., 2008). Looking back at the early functional studies for IL-18, the anti-inflammatory effect of neutralizing IL-18 was primarily observed in the lamina propria (Kanai et al., 2001; Siegmund et al., 2001). Kanai et al. (2001) demonstrated the main expression of IL-18 in their model in macrophages of the lamina propria. Here either deletion of macrophages or neutralizing IL-18 resulted in prevention of disease (Kanai et al., 2001). This would also allow for a clarification of the role of caspase-12. In the absence of caspase-12, one regulatory pathway of Nlrp3 inflammasome inhibition is missing, and thus active IL-18 is available to maintain the intestinal barrier at the level of IECs.

During the sustained DSS exposure, IL-18 affects the lamina propria and then the deleterious effect of IL-18 dominates the systems. During chronic DSS exposure, there is enough time to recover, and the caspase-12-meditated IEC proliferation results in a sufficient regeneration that becomes dangerous when the carcinogen enters the system. The working model suggests that activation of Nlrp3 and IL-18 at the level of IEC is critical for maintaining the intestinal homeostasis and thus in the end the barrier against bacterial translocation. In striking contrast, activation of Nlrp3 in the lamina propria promotes intestinal inflammation.

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Act1, Scene Brain: Astrocytes Play a Lead Role

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Interleukin-17 (IL-17) is crucial for the progression of experimental autoimmune encephalomyelitis. In this issue of *Immunity*, Kang et al. (2010) report that neuroectoderm-derived astrocytes are the critical cellular element that responds to IL-17.

Act1 is a crucial adaptor protein in the interleukin-17 receptor (IL-17R) signaling complex. Upon IL-17 binding, the heteromeric transmembrane receptor (IL-17RA and IL-17RC) recruits and interacts with Act1 by its SERIF domains (Chang et al., 2006). A variety of cells express the IL-17R signaling complex, facilitating inflammatory responses to locally produced IL-17 in most tissues (Gaffen, 2009). IL-17-responsive cells are capable of expanding local tissue inflammation because IL-17 induces Act1-dependent upregulation and mRNA stabilization of proinflammatory cytokines and CXC chemokines (Hartupee et al., 2007).

Several inflammatory autoimmune disease models including experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis (MS), have been shown to be mediated to a substantial extent by Th17 cells, although both Th1 and Th17 cells are capable of inducing EAE individually, but probably by different mechanisms and with different efficiency (Qian et al., 2007). As previously reported (Qian et al., 2007), mice deficient in Act1 exhibited delayed onset and less severe myelin oligodendrocyte glycoprotein (MOG) peptide-induced EAE. Given that there are multiple stages and cell types involved in EAE pathology, it was unclear at which stage of disease pathogenesis IL-17, signaling via Act1, was necessary, and it is this question that was approached by Kang et al. (2010) in this issue of *Immunity*.

EAE is a multistep inflammatory process initiated by Th1 and Th17 cells. During actively induced EAE, myelin-specific Th1 or Th17 cells are activated and expand in the peripheral lymphoid tissues in response to myelin peptide and complete Freund's adjuvant (CFA) immunization. These activated T cells extravasate across the blood brain barrier (BBB) secondary to interaction of very late activation antigen 4 (VLA-4) expressed on activated T cells with vascular cell adhesion molecule (VCAM) expressed on cerebrovascular endothelial cells (Yednock et al., 1992). In the central nervous system (CNS), infiltrating myelin-specific T cells must be reactivated by brain-resident antigen-presenting cells (including microglia, macrophages, and myeloid dendritic cells) presenting myelin antigen (Bailey et al., 2007; Tompkins et al., 2002). Release of Th1 or Th17 cell cytokines induced by this interaction results in local inflammation and demyelination of white matter tracts, reducing the ability of axons to conduct electrical signals and eventually neuronal transection. Astrocytes are involved in nearly all processes within the brain, but their contribution to EAE